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— with international search report(71) Applicant (*for all designated States except US*): ROCHE DIAGNOSTICS GMBH [DE/DE]; 68298 Mannheim (DE).(88) Date of publication of the international search report:
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: THERMOSTABLE ENZYME PROMOTING THE FIDELITY OF THERMOSTABLE DNA POLYMERASES- FOR IMPROVEMENT OF NUCLEIC ACID SYNTHESIS AND AMPLIFICATION *IN VITRO*

Error rates of different DNA polymerases in PCR

Polymerase	Template conc. (ng)	yield (ng)	DNA duplication d	blue colonies lacI	white colonies lac ⁺	total number of colonies	% lac ⁺	error rate (1/seq)
Taq Ch.	10	11650	10.2	130	2261	2391	6.4	1.57 x 10 ⁻⁴
Hifi Ch.	10	11560	10.2	40	6458	6498	0.72	2.06 x 10 ⁻⁴
Pwo	10	9675	9.9	17	6891	6908	0.29	8.32 x 10 ⁻⁴
Taq/Exo 1	10	11550	10.2	94	4291	4385	2.14	6.10 x 10 ⁻⁴
Taq/Exo 2	10	11125	10.1	146	7644	7790	1.87	6.36 x 10 ⁻⁴
Taq/Exo 3	10	8500	9.7	133	8168	8321	1.6	4.74 x 10 ⁻⁴
Taq/Exo 4	10	1282	7	79	7236	7315	1.08	4.44 x 10 ⁻⁴
Taq/Exo 6	10	238	4.6	25	2674	2724	0.92	1.16 x 10 ⁻⁴

* Due to the unfavorable ratio of Taq/Exo the product yield was low. This results in an apparently low amplification efficiency d, which is an important parameter in the formula used for the calculation of the error rate.

(57) Abstract: A purified thermostable enzyme is derived from the thermophilic archaebacterium *Archaeoglobus fulgidus*. The enzyme can be native or recombinant, is stable under PCR conditions and exhibits double strand specific exonuclease activity. It is a 3'-5' exonuclease and cleaves to produce 5'-mononucleotides. Thermostable exonucleases are useful in many recombinant DNA techniques, in combination with a thermostable DNA polymerase like *Tag* especially for nucleic acid amplification by the polymerase chain reaction (PCR).

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP 00/09423

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/55 C12N15/54 C12N15/10 C12N9/22 C12N9/12
C12Q1/68 C12P19/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N C12Q C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data, PAJ, CAB Data, STRAND, EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	H.P. KLENK ET AL.: "Archaeoglobus fulgidus section 43 of 172 of the complete genome" EMBL SEQUENCE DATABASE, 1 December 1997 (1997-12-01), XP002139308 Hinxton, UK cited in the application	1-3
Y	Accession no. AE001064, TrEMBLrel 029675, 01-01-1998; EXODEOXYRIBONUCLEASE III (XTHA); — —/—	17,18

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *S* document member of the same patent family

Date of the actual completion of the international search

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International application No
PCT/EP 00/09423

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"Archaeoglobus fulgidus genome" THE INSTITUTE FOR GENOME RESEARCH; TIGR DATABASE, 15 May 1999 (1999-05-15), XP002139309 US cited in the application	1-3
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/09423

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ZHU Y S ET AL: "THE USE OF EXONUCLEASE III FOR POLYMERASE CHAIN REACTION STERILIZATION" NUCLEIC ACIDS RESEARCH,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 19, no. 9, 28 January 1991 (1991-01-28), page 2511 XP000199115 ISSN: 0305-1048 page 2511, left-hand column, line 21 - line 26 page 2511, right-hand column, line 3 - line 5	17,18
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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/EP 00/09423

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SMITH C ET AL: "GENERATION OF COHESIVE ENDS ON PCR PRODUCTS BY UDG-MEDIATED EXCISION OF FU, AND APPLICATION FOR CLONING INTO RESTRICTION DIGEST-LINEARIZED VECTORS" PCR METHODS & APPLICATIONS,US,COLD SPRING HARBOR LABORATORY PRESS, vol. 2, no. 4, 1 May 1993 (1993-05-01), pages 328-332, XP002071999 ISSN: 1054-9803 the whole document	
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/09423

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☒ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☒ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: (1-3,5-16)-complete, 18-partially

Thermostable enzyme exhibiting 3'-exonuclease-activity but essentially no DNA polymerase activity whereas this enzyme enhances fidelity of an amplification process when added to a second enzyme exhibiting polymerase activity; said enzyme obtainable from *Archaeoglobus fulgidus*, whereas this enzyme is able to cooperate as proofreading enzyme with a second enzyme exhibiting polymerase activity; Composition comprising a first thermostable enzyme exhibiting 3'-exonuclease-activity but essentially no DNA polymerase activity and a second enzyme exhibiting DNA polymerase activity whereas the fidelity of an amplification process is enhanced by the use of the composition to the use of the single second enzyme; a method of preparing or amplifying DNA using said composition; said method whereas the mixture of a -first thermostable enzyme exhibiting 3'-exonuclease-activity but essentially no DNA polymerase activity and -a second enzyme exhibiting DNA polymerase activity produces PCR products with lower error rates compared to PCR products produced by the second enzyme exhibiting DNA polymerase activity in absence of the first thermostable enzyme exhibiting 3'-exonuclease-activity but essentially no DNA polymerase activity; said method whereas PCR products with blunt ends are obtained; a method for amplifying DNA using said thermostable enzyme exhibiting 3'-exonuclease-activity which enzyme is not or only to a negligible extend active on linear single stranded DNA;

2. Claims: 4-complete, 18-partially

Thermostable enzyme exhibiting 3'-exonuclease-activity but exhibits reduced DNA polymerase activity; a method for amplifying DNA using said thermostable enzyme exhibiting 3'-exonuclease-activity which enzyme is not or only to a negligible extend active on linear single stranded DNA;

3. Claims: 17-complete, 18-partially

A method for amplifying DNA using a thermostable enzyme exhibiting 3'-exonuclease-activity which enzyme is not or only to a negligible extend active on linear single stranded DNA;

INTERNATIONAL SEARCH REPORT

International Application No
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